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TITLE: Obstructing Androgen Receptor Activation in Prostate Cancer Cells Through Post-translational Modification by NEDD8

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14. ABSTRACT The overall goal of this study is to investigate the effect of post-translational NEDD8 modification on androgen receptor. In year 3, we proposed to establish Jab1 shRNA expressing lines of prostate cancer cells and to characterize the effects of Jab1 silencing on prostate cancer cell growth and proliferation. Several stable cell lines have been established, and experiments are being conducted to characterize these cell lines. These works are currently being continued into the final no-cost extension year.					
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Introduction

In this study, we have found that Jab1 physically interacted with AR *in vivo* and *in vitro*. Overexpression of Jab1 enhanced androgen-dependent transcriptional activation of AR. The JAMM motif of Jab1 is required for this enhancement, suggesting the involvement of Jab1's isopeptidase activity. Endogenous neddylated AR was accumulated in Jab1 knockdown cells and NEDD8 was rapidly removed upon androgen administration. We found a potential E3 ligase that catalyzed this modification and inhibited AR's transcriptional activity. More significantly, PR and ER were also susceptible to PIASy-mediated neddylation and inhibition, suggesting a likely new mechanism that modulates nuclear receptor's activity. Our results linked neddylation and de-neddylation pathways with regulation of AR and other nuclear receptor's function. We hypothesize that AR is subject to modification by NEDD8, and this process may be a therapeutic target to control prostate cancer growth.

Body

In year 3, we proposed to conduct Specific Aim 4 in the original proposal. In Aim 4, we proposed to determine the effects of silencing endogenous Jab1 in the growth of prostatic cancer cells.

In Aim 4-1, we proposed to generate prostatic cancer cell lines with expression of shRNA against Jab1 or scramble control shRNA. DNA encoding the shRNA sequences against endogenous Jab1 were cloned into the LentiLox 3.7 vector under the control of the U6 promoter. The Jab1 shRNA LentiLox 3.7 vector was mixed with the ViralPower™ Packaging Mix (containing pLP1, pLP2 and pLP/VSVG DNAs) and used for transfection into 293T cells. After viral particles were packaged and released, culture media were harvested to infect LNCaP and PC3 cell lines and silencing effect by shRNA were determined by detecting the diminishing protein levels of endogenous Jab1. Furthermore, the mRNA and protein levels of PSA or Probasin, which are known AR target genes, were characterized after Jab1 silencing.

This aim was described in Task 4-1, which was scheduled to be completed in months 24-27. We planned to generate prostatic cancer cell lines with expressing of shRNA against Jab1 and scramble control. Ultimately, stable cell lines with Jab1 expression inhibited will be established.

We are pleased to report that several Jab1 shRNA expressing lines have been successfully established. These cell lines are currently being used to characterize their effects on Jab1 expression and for use in conducting experiments described in Aim 4-2.

In Aim 4-2, we proposed to characterize the effects of endogenous Jab1 silencing on protein stability of p53 and p27 and cell growth. The effects of Jab1 silencing on the cell proliferation were determined by MTT assay. We plated both LNCaP and PC3 with Jab1 silencing or control at 1×10^4 cells per well in a 96 well microtiter plate, and the effects on cell proliferation and growth curve are currently being analyzed. We expect

that silencing endogenous Jab1 will impede on cell proliferation of both LNCaP and PC3 cells. To understand growth inhibition mediated by Jab1 silencing, cell cycle analyses will also be conducted to determine which phase of cell cycle was arrested. Sample will be collected and subjected to FACS flow cytometer analysis. In addition, we will measure the steady state protein level of p53, p21, p27, and pRb. In the Jab1 silencing cell lines, we expect protein level of p53, p21 and p27 will be accumulated and pRB will be hypo-phosphorylated.

This Aim was described in Task 4-2 to be completed in months 27-36: Characterize the effects of endogenous Jab1 silencing on protein stability of p53 and p27 and cell growth. Ultimately, we will demonstrate evidence to suggest whether Jab1 affects protein stability of p53 and p27 and cell growth.

This Aim is currently being conducted in the no-cost extension period.

Key Research Accomplishments:

Year 3:

- We have established several Jab1 shRNA expressing cell lines in LNCaP and PC3 cells.
- We have partially characterized the effect of Jab1 silencing on cell growth and proliferation of prostate cancer cells.

Reportable Outcome:

- A new postdoc, Dr. Han-Fu Cheng, was recently recruited to continue on this project during the no-cost extension year.

Conclusion:

In summary, we have completed part of the proposed tasks in the third year. We expect to continue the research into the forth year. No changes on future work are necessary.

References:

None

Appendices:

None

Supporting Data:

None